Early stage fruit analysis to detect a high risk of bitter pit in ‘Golden Smoothee’

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Running title: Bitter pit prediction in ‘Golden’ apples based on early fruitlet analysis

ABSTRACT

Fruit mineral analysis at harvest is recommended as a predictive method to assess the risk of bitter pit (BP) in apple orchards, although it only provides valuable information if conducted just before harvest. To gain more time to implement corrective action, some studies proposed early season analysis of fruitlets. However, neither results were reported for analysis accuracy, nor the best time to perform it. The objective of this study was to evaluate the accuracy of early season fruitlet analyses at different stages — 40, 60 and 80 days after full bloom (DAFB) — to predict BP in 'Golden Smoothee' apples. Multivariate models for each early stage were developed and compared to a linear model using the calcium (Ca) content alone. Both the multivariate analyses and linear correlations suggested 60 DAFB as the best time to perform early mineral analysis. The Ca concentration in the fruit contributed greatly to BP incidence either at an early stage or at harvest. The boron (B) concentration showed a negative correlation with Ca concentration and a positive correlation with BP incidence. The other tested nutrients (magnesium, nitrogen, potassium) showed little effect on the prediction models and/or an irregular pattern. The accuracy of the multivariate model (R^2 = 0.580) was not significantly better than the analysis of Ca alone (R^2 = 0.504) when the occurrence of BP was high. Finally, a Ca threshold at 60 DAFB equal to or greater than 11.0 mg 100 g^{-1} fresh weight (f. w.) indicated a low risk of BP (< 10% of incidence). This early season threshold value was a better indicator of the BP risk than the traditional threshold value at harvest (5-6 mg Ca 100 g^{-1} f. w.).

KEYWORDS: *Malus domestica*, Calcium Disorders, Early Fruitlet Analysis, Nutrient balance, Quality prediction.

INTRODUCTION

Bitter pit (BP) is a disorder in apples (*Malus*×*domestica* Borkh.) that is closely related to calcium (Ca) nutrition, as well as to other nutrients such as boron (B), magnesium (Mg), nitrogen (N), or potassium (K) (Casero et al., 2004; Ferguson et al., 1999). In general, high Ca and B contents are related to a low incidence of BP, while high incidence is positively associated with N, K and Mg. The incidence of BP can fluctuate seasonally and even in the same orchard (Lotze et al., 2006; Torres et al., 2015 and 2017). Because of the absence of an effective control method for BP, some studies have focused on prediction. Effective BP prediction allows optimizing control measures (e.g., increasing Ca sprays, or decreasing N and K fertilization), or helping the packing house in choosing the right management approach (Manganaris et al., 2005; Torres and Alegre, 2012; Torres et al., 2015). Methods such as inducing symptoms before they naturally occur, like for instance 'Mg infiltration' (Burmeister and Dilley, 1993; Retamales et al., 2000), 'ethephon dips' (Eksteen et al., 1977) or the 'passive method' (Torres et al., 2015), can predict BP 30-10 days before harvest. However, these methods do not provide enough time for growers to take new actions to control BP.

Another predictive method that has been used for years to assess the risk of BP is based on fruit mineral analysis at harvest for macronutrients, such as Mg, K, N, and especially, Ca contents (Autio et al., 1986; Ferguson and Triggs, 1990; Wolk et al., 1998). Sharples (1979) documented fruit mineral standards for 'Cox's Orange Pippin' apples, suggesting that the behaviour of fruit in storage can be predicted from an analysis at harvest. Terblanche et al. (1980) and Waller (1980) reported that balanced levels of Ca with other minerals (N, Mg, K) ensured the absence of BP in fruit. Currently, most of these thresholds are still used for all varieties of apples (Lotze et al., 2008; von Bennewitz et al., 2015). However, these ratios can only provide valuable information just before harvest. Some authors have suggested early season analysis of fruitlets in order to gain time and then implement corrective action if a BP risk is present (Brooks, 2001; Conway et al., 1994; Drahorad and Aichner, 2001; Ferguson and Triggs, 1990). The concept of early season fruitlet analysis has been previously investigated by other authors to predict post-harvest fruit quality attributes, but its relationship with BP has not been studied (Fallahi et al., 1985; Marcelle et al., 1989).

The hypothesis that early analysis can be a useful tool to predict BP is based on the strong relationship between the Ca concentration in fruitlets at the early stage and in fruit at harvest. Brooks (2001) reported that the Ca content in a 50 g fruitlet reflected the Ca content at harvest and, consequently, predicted BP, but he did not determine the statistical accuracy of the proposed approach. Drahorad and Aichmer (2001) reported that the K/Ca ratio in fruit at harvest can be predicted at early stages and, consequently, could provide useful information about the risk of BP and other physiological disorders, but they did not report information about the precision of the model. The results of Brooks (2001) and Drahorad and Aichmer (2001) were obtained from fruitlets with an average weight of 50 g and 70 g, respectively, which are approximately equivalent to 80 and 90 days after full bloom (DAFB) under our growing conditions, respectively. Nevertheless, Lotze et al. (2008) found a better correlation using mid-season fruitlets (41-82 DAFB) than those of late season (83-118 DAFB). Those results were presented as preliminary, but such research has not been further reported. Hence, there are no current references that clearly indicate the best sampling date for BP prediction based on an early stage mineral analysis, which is a critical point related to method accuracy.

The objective of this study was to evaluate the accuracy of early season fruitlet analyses at different times (40, 60, and 80 DAFB, and at harvest) as a predictive tool for post storage BP in 'Golden Smoothee' apples from a semi-arid region (Ebro Valley, Spain).

2. MATERIAL AND METHODS

2.1. Plant material

Experiments were carried out in Lleida (NE Spain), over three consecutive seasons in 10 commercial apple orchards of ‘Golden Smoothee’ with different levels of BP susceptibility. All of the orchards studied were mature, and their trees were normally spaced (approximately 4 × 1.2 m), grafted onto M9 rootstock, and fully irrigated. They were all subjected to standard cultural practices of pruning, fertilization, irrigation and crop management.

2.2. Fruit sampling

For mineral analysis, 20-fruit samples were collected from each orchard at 40, 60, and 80 DAFB, and at commercial harvest when the starch index was between 7 and 8 (starch chart EC-Eurofru). In each orchard, 20 trees that showed equally vigorous growth were selected. An apple was taken from each tree at 130-170 cm height above the ground and from spurs on 2-year-old shoots. Another 80-fruit sample was collected from each orchard at harvest to evaluate the incidence of post-harvest BP.

2.3. Mineral analysis

Fruit for mineral analyses were carefully washed, and two longitudinal slices were cut from the opposite sides of each fruit, excluding the core and seeds. The complete sample from each group was weighed, dried at 75 °C to constant weight, and then re-weighed to determine the dry mass percentage. The dried tissue was ground, and a sub-sample was wet-digested with concentrated nitric acid and hydrogen peroxide in a microwave oven (Milestone MCR 6E, Bergamo, Italy). The nutrient concentrations were determined according to standard procedures (AOAC, 2006). We focused on nutrients related to BP according to literature review: B, Ca, Mg, N and K. The B, Ca, Mg and K concentrations were determined using inductively coupled plasma-optical emission spectroscopy (ICP-OES). The N concentration was determined via Kjeldahl analysis.

2.4. Bitter pit evaluations

The 80-fruit sample collected from each orchard at harvest were placed in cold storage at 0 ºC and at 90% relative humidity (RH). After four months in cold storage, samples were transferred at 20 ºC and 45% RH for 7 days. Immediately after that, all of the apples contained in each sample were individually examined for any external signs of superficial BP symptom. The incidence of BP of each sample was calculated as the percentage of fruit with BP symptoms.

2.5. Data Analysis

Chemometric Analysis

Principal component analysis (PCA) of all of the measurements taken during the three seasons was performed (without including the BP incidence) to provide exploratory data analysis and a visualisation of all the data set information. The purpose of the PCA was to detect various clusters in terms of BP occurrence and to determine which variables were related to each other, as well as classification of the samples. Then, partial least squares regression (PLS) was calculated at each sampling time (40, 60, 80 DAFB and harvest) for the three seasons (2010, 2011, and 2012) and for the different clusters detected by the PCA model. The accuracies of the models (R^2) were compared to designate the best time to perform early mineral analysis of fruitlets for BP prediction.

In all cases, data were centred and weighted with the inverse of the standard deviation of each variable to allow all the variables the same chance to affect the estimates of the components. The data were represented in terms of the first two principal components (PCs), which represent the most important portion of the variability of the data (Johnson and Wichern, 2007). The software used was The Unscrambler® (Version 10.4; Camo Process AS, Oslo, Norway).

Linear regression

The linear correlation was calculated using the Ca concentration in the fruit as the independent variable, and the percentage of BP after storage as the dependent variable. The correlation was calculated at each early stage time (at 40, 60, 80 DAFB) and at harvest. The significance level and R^2 value were calculated for each linear regression model. The data were analysed using the JMP® statistical software package (Version 8.0.1; SAS Institute Inc., Cary, North Carolina).

3. RESULTS AND DISCUSSION

3.1. Sources of bitter pit variability

Two PCs described 54% of the total variance (a third PC explained approximately 20% more) in the PCA model that was carried out to detect different clusters according to BP occurrence. Two groups of samples were clearly differentiated on the PC1 axis in the scores plot. One group corresponded to samples from 2012 and had a low BP occurrence (only 5% of the orchard showed BP symptoms), and a second group corresponded to samples from 2010 and 2011, and had a high occurrence of BP (90% of the orchards were affected) (Figure 1).

In the loadings plot, the measurements at 40 DAFB and 60 DAFB showed a strong effect on PC1, especially the Ca (negative), Mg (negative), and N (positive) concentrations; by contrast, Ca at 80 DAFB was closer to the origin, which suggested a lesser effect in the model (Figure 1). Finally, the results of the scores plot (in which two clusters can be differenced by PC1 according to the frequency of BP and the results of the loadings plot, in which measurements at 40 and 60 DAFB have a strong effect on PC1) suggested that the best sampling date to predict BP was between 40 to 60 DAFB.

According to the loadings plot, the Ca concentration seemed to be negatively correlated with B, independently of the sampling date (Figure 1). These results are in line with those of Benavides et al. (2002), who observed a negative correlation between Ca and B at harvest, with a multivariate analysis that evaluated the relationships between textural parameters, quality attributes, and mineral elements in ‘Golden Smoothee’ apples. A relationship between Ca at harvest, and at 40 or 60 DAFB, was also showed by the loading plot. These results coincide with other studies regarding BP prediction and Ca content at harvest through an early stage analysis (Brooks, 2001; Conway et al., 1994; Ferguson and Triggs, 1990).

3.2. PLS models

3.2.1. PLS at harvest

The accuracy of the PLS model at harvest was R^2 = 0.424 for two PCs (Table 1). Increasing the number of PCs did not significantly improve the accuracy (data not shown). The Ca and Mg concentrations contributed negatively to the BP incidence, especially for Ca, which had the largest (negative) coefficient. On the other hand, the B, N, and K concentrations contributed positively to BP incidence, as indicated by similar weighted regression coefficients (Figure 2).

According to the literature, BP is more common when a B deficiency is present, since B improves Ca movement to the fruit and the cell wall structure, as well as maintaining membrane stabilization (Granelli et al., 1989; Rosenberger et al., 2004; Wojcik et al., 1999; Wojcik and Cieslinski, 2000). However, our results suggested the opposite, showing an increase in BP when a B surplus existed. This was confirmed by the PCA model mentioned above, in which Ca and B concentrations were negatively correlated. The role of B in plant physiology is unique among other nutrients because of the small difference that exists between deficiency and excess (Nable et al., 1997; Paparnakis et al., 2013). In this study, the B levels in the fruit could be considered to be excessive in most of the orchard, according to the recommendations in Peryea and Drake (1991), who considered excessive > 40 ppm of B in fruit. The reported symptoms of B toxicity are internal necrosis in fruit and stems (Nable et al., 1997), but BP symptoms are not generally present. Perhaps, the association of B with BP prediction may be indirectly related to Ca uptake or transport into the fruit. Recent studies have shown a relationship between xylem functionality, the Ca content, and the BP incidence in apples (Miqueloto et al., 2014). This evidence suggests that high levels of B could reduce the xylem functionality in the fruit stem — B toxicity is related to internal necrosis in the fruit or stems — and, consequently, reduce Ca uptake into the fruit, triggering the development of BP. However, this hypothesis has not been tested and more studies are needed to understand the relationship of B and Ca nutrition, as well as for BP incidence, especially in arid or semi-arid climates.

The other tested nutrients (K, Mg and N) showed expected behaviour (Figure 2). In general, apples with higher K, Mg and/or N levels at harvest are more likely to exhibit BP. The effect of K and Mg in the development of BP has been explained by their antagonist effect with Ca. High N level effects may trigger faster cell expansion and rapid fruit growth, leading to a reduction in the fruit Ca content and, consequently, fruit susceptibility to BP (De Freitas et al., 2015; Ferguson and Watkins, 1989; Saure, 2005).

3.2.2. PLS at 40 DAFB

The variance explained by the PLS model at 40 DAFB using two PCs had a $R^2 = 0.389$, slightly less than at harvest (Table 1). Additional PCs did not significantly improve the accuracy of the model. The Ca concentration at 40 DAFB contributed negatively to the BP incidence and had the largest (negative) coefficient. The B and K concentrations were also significant and contributed positively to BP. The N concentration had a slight negative effect, whereas the effect of Mg was not significant (Figure 2). As previously mentioned, the positive effect of N on the BP incidence may be related to rapid fruit growth; therefore, subsequent observations could show a positive effect when the growth rate of the fruit is larger.

3.2.3. PLS at 60 DAFB

The variance explained by the PLS model at 60 DAFB for two PCs had a $R^2 = 0.464$, which indicated that this was a better sampling time than 40 DAFB, and even better than at harvest (Table 1). The accuracy of the model did not improve when the number of PCs was increased. The Ca concentration, again, had the largest (negative) weighed regression coefficient, whereas the B and K concentrations contributed positively to the BP. The weight of the Ca concentration was almost twice that of the K concentration, suggesting a larger effect of Ca in the model than for K and the other nutrients. The N and Mg concentrations produced positive and negative effects, respectively, in the model, although they were not significant in comparison to the other nutrients (Figure 2).

3.2.4. PLS at 80 DAFB

The predictive power of a PLS model with measurements at 80 DAFB was lower than using harvest measurements, or even 40 DAFB and 60 DAFB, and was independent of the number of PCs. The optimum number of PCs was three, one more than at 40 DAFB or 60 DAFB, and had a $R^2 = 0.321$, whereas with two PCs had a $R^2 = 0.117$ (Table 1). The B concentration contributed strongly (positive) in the model, while the influence of the rest of the variables was minor (Figure 2). The possible causes of this lack of influence could be the instability of the Ca content in the fruit during this period, since is a period with high leaf transpiration and high shoot and fruit growth rates, especially in a semi-arid region (Lakso et al., 1999). Deviations within few days during this period could cause a great variability in the Ca concentration and, consequently, meaningless results. These could be corrected just before harvest, when the growth rate is reduced and the Ca concentration in fruit is stabilized.

These results suggested that an early mineral analysis of fruitlets at 60 DAFB was the best approach to predict BP incidence at early stages of 'Golden Smoothe' apples. This coincides with Lotze et al. (2008), who reported a better correlation with BP incidence for the Ca concentration in fruit at 41-81 DAFB than at 83-118 DAFB.

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The regression coefficients for the B concentration — expressed as mg 100 g^{-1} of dry weight (d. w.) in fruit — and Ca, Mg, N and K concentrations — expressed as mg 100 g^{-1} of fresh weight (f. w.) in fruit — as well as the regression coefficient for the intercept (B_0), and coefficient of determination (R^2) for each model, are shown in Table 1. However, we must note that the accuracy of the models can vary within cultivar and region.

3.3. PLS model from HBP seasons

The sample grouping in the PCA model — one group corresponding to a season with a low occurrence of BP (LBP) and a second group corresponding to seasons with high occurrences of BP (HBP) — suggests that seasonal factors (e.g., weather conditions) could contribute to the differences either in the mineral fruit composition or in the BP incidence. These outcomes are also supported by our previous studies that showed that either BP or mineral content were strongly related to the season of growth (Torres et. al., 2015 and 2017). Considering this, we separately studied the HBP samples to improve the accuracy of the BP prediction. We discarded a PLS model with data from the LBP season that had a low number of observations (10 orchards), and consequently yielded erratic results.

The R^2 of the models at 40 DAFB (0.390), 60 DAFB (0.580) and at harvest (0.574) slightly improved with respect to the data set from the three seasons (Table 2). By contrast, the accuracy of the model at 80 DAFB (R^2 = 0.108) was lower than that of the model for the three seasons (Table 2). Consequently, according to our results, a model for the prediction of BP based on mineral analyses of fruitlets at 80 DAFB could not be recommended for our growing conditions as Lotze (2008) suggested.

The goodness-of-fit values of the HBP models were similar to the model that used the three-seasons data set. However, the weight of B (positive) and K (negative) in the HBP model at 40 DAFB decreased and increased, respectively, compared to the three-seasons model. On the other hand, the regressions of the weighted coefficients of the HBP model at 60 DAFB were similar to those of the three-seasons model — the weight of Ca was twice that of the N or K concentration — which suggested greater reliability than at 40 DAFB (Figure 3). The weight of the B, Mg, N and K at harvest was not significant in comparison to the Ca weight (Figure 3). This was a significant change with respect to the three-seasons model in which all of the tested nutrients showed a relevant weight in the model, in agreement with the suggestions reported by Ferguson et al. (1999; 1990). They reported that Ca in fruit at harvest was the primary determinant of BP risk, whereas Mg and K may be useful only in specific cases where the Ca vs. BP relationship did not fit the expected pattern.

3.4. Bitter pit prediction through Ca content

According to the various PLS models, Ca seemed to be the nutrient that had the largest contribution to BP incidence, which suggested that Ca concentration alone could be sufficient to predict BP in most cases, as reported by Ferguson et al. (1999; 1990) for analysis performed at harvest. We calculated the linear regressions between BP and the Ca content in fruit at early stages and at harvest to confirm this observation. Some authors have also suggested that the N/Ca, K/Ca and/or Mg/Ca ratios might predict BP (Casero et al., 2010; De Freitas et al., 2015; Drahorad and Aichner, 2003; Miqueloto et al., 2014; Van der Boon, 1980). However, our results using these ratios were negative and inconsistent (data not shown), in agreement with Ben (2006) and Ferguson et al. (1999; 1990), who did not observe an improvement in the BP correlation when using Ca ratios with other nutrients.

The linear correlations were significant for 40 DAFB and 60 DAFB (Table 3). However, the accuracy decreased in comparison to their respective PLS models. The accuracy at 40 DAFB in 2010 and 2011 was R^2 = 0.409 and 0.358, respectively, but decreased to R^2 = 0.151 when both HBP seasons were analysed together. The decrease in accuracy was lower at 60 DAFB, especially for the HBP seasons. The accuracy at 60 DAFB was R^2 = 0.618 and 0.742, in 2010 and 2011, respectively, and decreased to R^2 = 0.503 when both seasons were analysed together (Table 3). This suggested that although the Ca concentration in fruit is strongly correlated with BP, other triggers, different than mineral composition, may exist and could vary depending on the season. The linear correlation at 80 DAFB was non-significant, as expected according to our previous multivariate analyses (Table 3). Our results for 80 DAFB differed from the recommendation of Brooks (2001) or Drahorad and Aichmer (2001) who, used 50 g and 70 g of fruitlet, respectively, — these fruitlet weights were achieved at approximately 80-90 DAFB under our growing conditions — to analyse the Ca concentration and to predict the BP level. According to our results, 60 DAFB is the date recommended to perform a mineral analysis to predict BP based either on a PLS model or on the Ca concentration alone, as suggested by Lotze (2008). Sampling at 60 DAFB would allow growers to know the potential risk of BP development approximately 100 days before the expected harvest date. If a high risk of BP is detected at that moment, there would be enough time to take measures to reduce its incidence. According to our previous studies, increasing the number of sprays that occurred closer to harvest can improve BP control, and their combination with dips at post-harvest results in the most efficient way to reduce the BP incidence (Torres et al. 2017).

The accuracy of Ca alone was lower than that of the PLS model, especially when many orchards showed a low BP incidence. However, a threshold for Ca content to predict BP, at least qualitatively, will be more useful for farmers and advisors than a multivariate model. In general, the fruit mineral analysis approach is able to provide a threshold value above that the risk of BP increases. We observed that at 60 DAFB, Ca concentrations lower than 11.0 mg Ca 100 g^{-1} f. w. suggested a BP incidence higher than 10%, independently of the season, LBP or HBP, with a 73% chance of occurrence (Figure 4). This accuracy was better than that of the traditional standard of 5-6 mg Ca 100 g^{-1} f. w. in fruit at harvest (Terblanche et al., 1980; von Bennewitz et al., 2015), which had an accuracy of 57%, and similar to the 'passive method' (71%) recently proposed by us (Torres et al., 2015). However, the 'passive method' allows to know the risk of BP through an incidence scale of three degree (<5% or low; 5%-10% or medium; >10% or high). It is an easy and inexpensive way for predicting BP between 30-10 days before harvest, therefore, could be used after the early mineral analysis in order to improve prediction accuracy. A more effective prediction would help growers and packing houses to decide whether to increase the number of Ca sprays or to use $CaCl_2$ dips, which would mean an optimization of the BP control.

The proposed threshold seemed robust when tested with a data set from seven orchards in different seasons and from the same region, with an accuracy of 71%. Nevertheless, with data from other regions with weather conditions not as extreme as in the present study, the level of Ca at 60 DAFB did not achieve the proposed threshold and BP was apparent, although at an incidence lower than 10%. In this case, it was not possible to predict the BP incidence using a multivariate model (data not shown). According to this, many studies have found no relationships in seasons with a low frequency of BP (Autio et al., 1986; Hewett and Watkins, 1991; Le Grange et al., 1998; Van der Boon, 1980). In any case, Ca concentrations at 60 DAFB higher than 11.0 mg Ca would indicate a low risk of BP, whereas the prediction of a lower threshold depends on the region.

Under our growing conditions, there was a similar occurrence when we used fruit Ca at harvest to predict BP: values higher than 6 mg 100 g^{-1} f. w. indicate a low BP incidence, whereas at lower values, the BP incidence depends on the orchard and/or the year. Our previous studies indicated that a low Ca concentration in fruit (< 4 mg 100 g^{-1} f. w.) is not enough to develop BP (Torres et al., 2017). Some authors question whether the Ca nutrition is the main cause of BP and propose that abiotic stress situations could have a more important role than the Ca concentration on the mechanisms that trigger BP development (Autio et al., 1986; Krawitzky et al., 2016; Marcelle et al., 1989; Saure, 2014). Stress increases the production of reactive oxygen species, which cause lipid peroxidation with an increase in membrane leakiness, leading to rapid vacuolation of parenchyma cells and the loss of ions, such as apoplastic Ca. Therefore, a final deficiency of Ca could only be considered to be a result, but not a cause (Saure, 2014). However, the proper balance of Ca with the rest of the nutrients would help to reduce the susceptibility to BP by improving cell wall stability and membrane integrity (Saure, 1996; Saure, 2014; Witney and Kushad, 1987; Witney and Kushad, 1990), explaining the strong relationship between Ca in fruit and BP during some years. Future research must address the relationship between weather conditions and BP to gain a better understanding of BP triggers and improve the accuracy of the predictions.

4. CONCLUSIONS

The relationship between BP and the mineral concentration of nutrients that have a direct effect on BP (Ca, Mg, K, N) was examined using multivariate analysis (PCA and PLS models), and linear correlation at various early stages (40, 60, 80 DAFB) and at harvest. The correlations carried out using data at 40 and 60 DAFB were significant, whereas the correlations at 80 DAFB were non-significant, suggesting that 60 DAFB was the best time to perform early mineral analysis in fruitlets to predict BP due to a higher accuracy. Of all of the nutrients analysed, the Ca concentration in fruit contributed the most to BP incidence, either at early stages or at harvest. Other tested nutrients showed a smaller effect on the prediction models, and/or an irregular pattern, suggesting that the Ca content in fruit at early stages can be an indicator of the risk of BP incidence. Finally, our results showed a good chance of a low BP incidence (< 10%) when the Ca level at 60 DAFB was equal or higher than 11.0 mg 100 g^{-1} f. w., whereas for a lower Ca concentration, the diagnosis depended on the region or season. This threshold value early in the season was a better indicator of the BP risk than the threshold value at harvest, which is traditionally promoted (5-6 mg Ca 100 g^{-1} f. w.). The relationship between the mineral concentration in apples and BP continues to be a complex and poorly understood process, and its influence in triggering BP could be minor. Considering factors other than the nutritional balance as the main cause could provide a better understanding and more effective prediction and control of BP.

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5. FIGURES AND TABLES

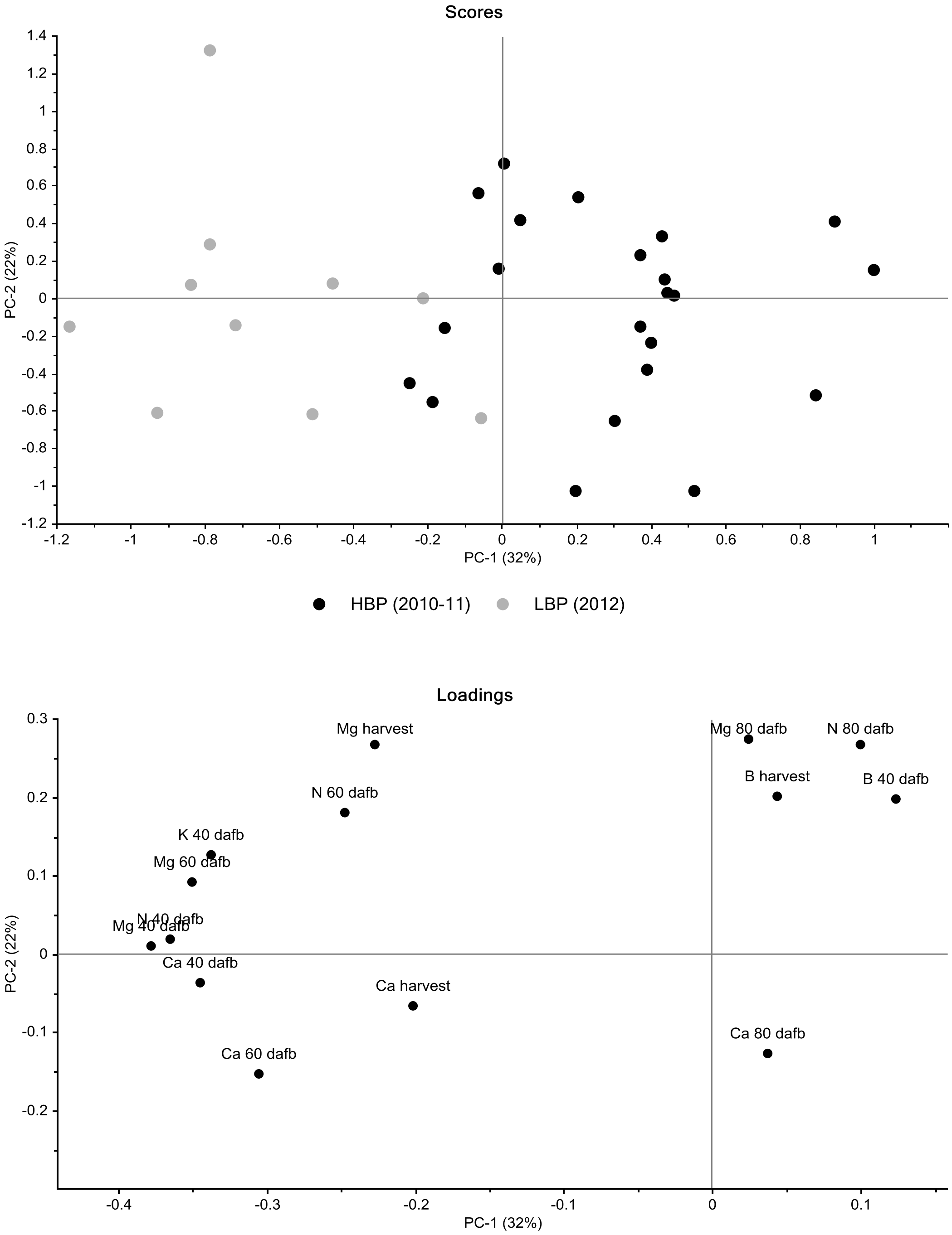

Figure 1. Score plot (above) and loading plot (below) of PC1 vs. PC2 from a full data PCA model. In the score plot, the samples are grouped according to the occurrence of bitter pit: LBP group corresponds to the samples (orchards) from 2012, with a low occurrence of BP (only 5% of the orchard showed BP symptoms), and HBP corresponds to samples from 2010 and 2011, with a high occurrence of BP (90% of affected orchards). In the loading plot, 20 variables were included: B, Ca, Mg, N and K at 40, 60, 80 days after full bloom (DAFB) and at harvest, respectively.

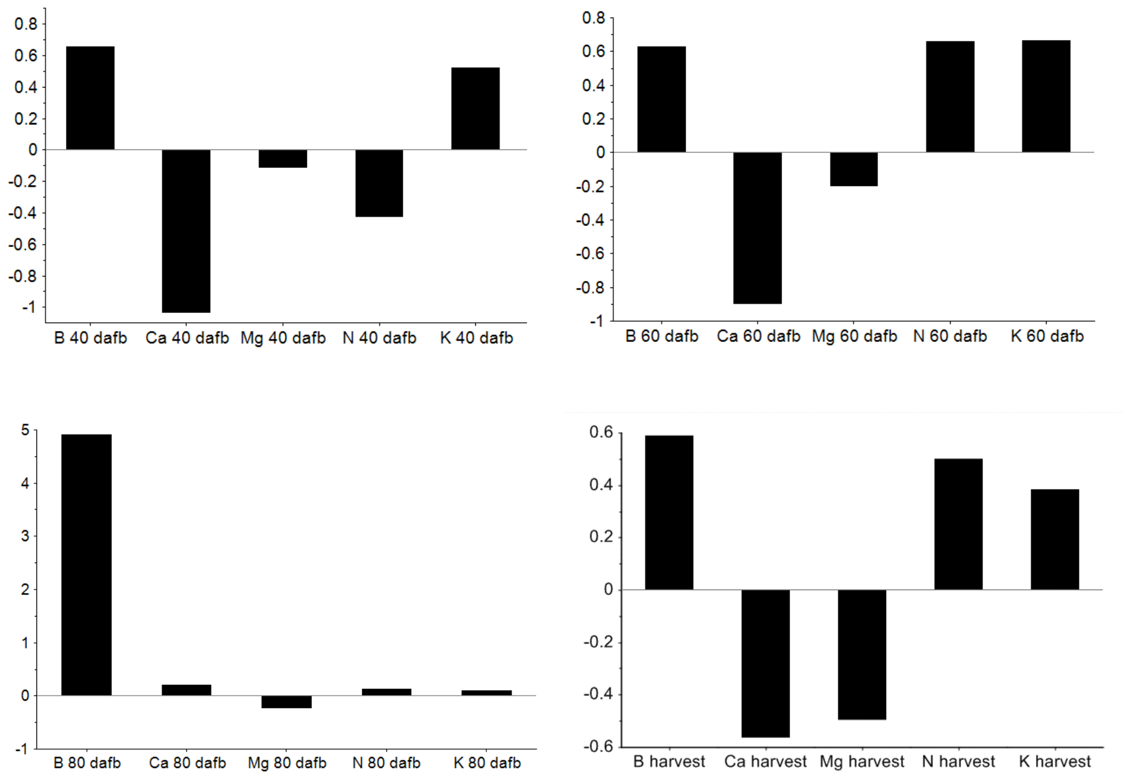

Figure 2. Weighted regression coefficients of the PLS models to predict bitter pit incidence at 40 days after full bloom (DAFB) (above-left), 60 DAFB (above-right), 80 DAFB (below-left) and at harvest (below-right), based on the B, Ca, Mg, N and K concentrations in fruit. Data set from three seasons (2010, 2011 and 2012).

Table 1. Regression coefficients (B_i) of the PLS models based on the B concentration (expressed as mg 100 g^{-1} of dry weight) and the Ca, Mg, N and K concentrations (expressed as mg 100 g^{-1} of fresh weight) in fruit, as well as the regression coefficient for the intercept (B_0), to predict bitter pit incidence at 40 days after full bloom (DAFB), 60 DAFB, 80 DAFB and at harvest. Data set from three seasons (2010, 2011 and 2012).

Sampling date	Num. PCs	B_0	B	Ca	Mg	N	K	R^2
40 DAFB	2	14.655	1.740	-0.627	-0.115	-0.038	0.075	0.389
60 DAFB	2	-14.859	2.597	-3.877	-0.885	0.266	0.226	0.464
80 DAFB	3	-24.350	4.923	0.2113	-0.235	0.138	0.099	0.321
Harvest	2	-33.827	5.003	-2.652	-0.432	0.474	0.223	0.424

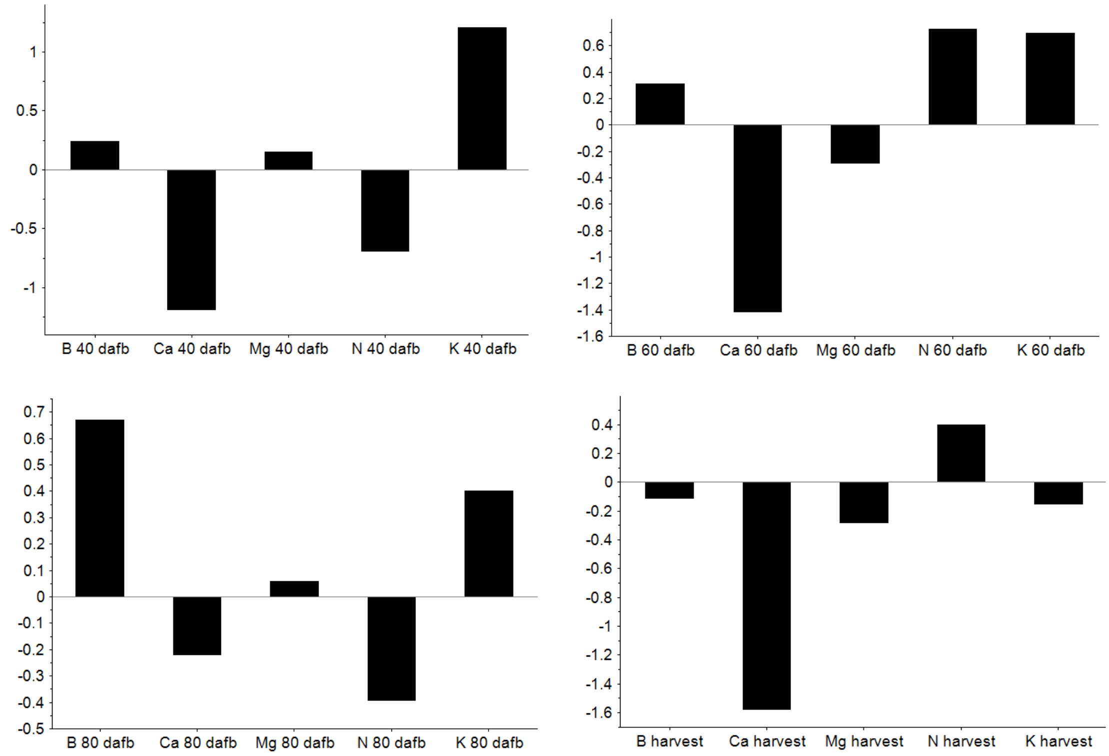

Figure 3. Weighted regression coefficients from the PLS models to predict bitter pit incidence at 40 days after full bloom (DAFB) (above-left), 60 DAFB (above-right), 80 DAFB (below-left) and at harvest (below-right) based on B, Ca, Mg, N and K concentrations in fruit. Data set from 2010 and 2011 seasons (high occurrence of bitter pit: 90% of orchard affected).

Table 2. Regression coefficients (B_i) of the PLS models based on the B concentration (expressed as mg 100 g^{-1} of dry weight) and the Ca, Mg, N and K concentrations (expressed as mg 100 g^{-1} of fresh weight) in fruit, as well as the regression coefficient for the intercept (B_0), to predict the bitter pit incidence at 40 days after full bloom (DAFB), 60 DAFB, 80 DAFB and at harvest. Data set from 2010 and 2011 (high occurrence of bitter pit: 90% of orchard affected).

Sampling date	Num. PCs	B_0	B	Ca	Mg	N	K	R^2
40 DAFB	2	14.124	0.562	-1.623	0.311	-0.091	0.204	0.390
60 DAFB	2	26.548	0.879	-5.333	-1.565	0.248	0.158	0.580
80 DAFB	2	16.477	2.727	-1.111	0.344	-0.192	0.092	0.135
Harvest	2	60.589	-0.532	-6.050	-2.068	0.220	-0.040	0.574

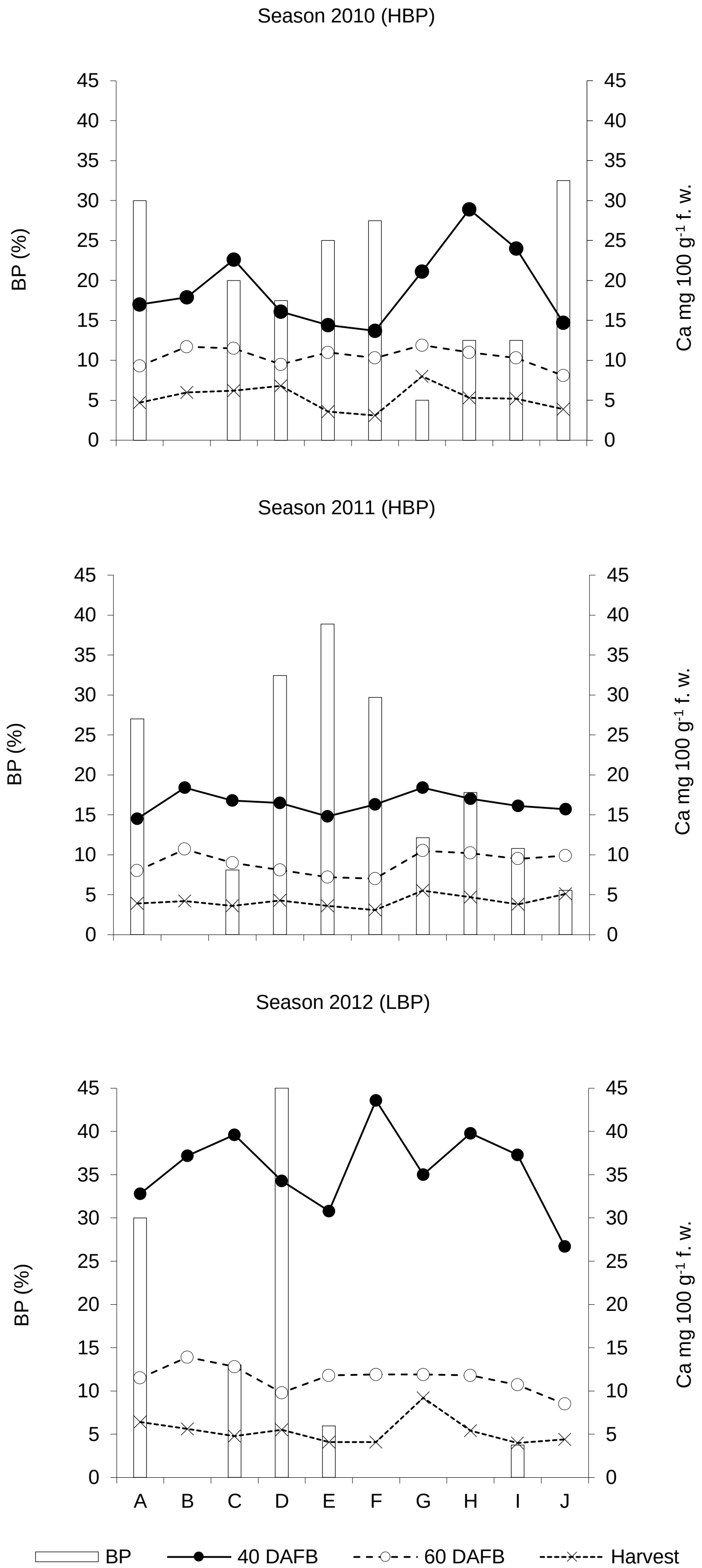

Figure 4. Bitter pit (BP) incidence for each orchard (A-J), and the Ca concentrations in the fruit (Ca mg 100 g^{-1} of fresh weight) at 40 and 60 days after full bloom (DAFB) and at harvest for each season.

Table 3. Linear correlation of the Ca concentration in fruit at 40, 60, 80 days after full bloom (DAFB) and at harvest with the bitter pit incidence.

Sampling date	2010		2011		2012		2010-11 [1]		2010-12	
	R^2	P	R^2	P	R^2	P	R^2	P	R^2	P
40 DAFB	0.409	*	0.358	*	0.026	n.s.	0.151	*	0.110	*
60 DAFB	0.618	**	0.742	**	0.105	n.s.	0.504	**	0.274	**
80 DAFB	0.282	n.s.	0.011	n.s.	0.134	n.s.	0.055	n.s.	0.022	n.s.
Harvest	0.557	**	0.180	n.s.	0.010	n.s.	0.218	*	0.231	**

* Correlation significant at the $P < 0.05$. ** Correlation significant at the $P < 0.01$

[1] Seasons with a high occurrence of bitter pit (90% of orchard affected).

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